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(54) Title: THE USE OF BACTERIAL PHAGE ASSOCIATED LYTIC ENZYMES TO PREVENT FOOD POISONING

(57) Abstract: The present invention discloses a method and composition for the treatment of bacterial contamination of food by the use of a phage associated lysing enzyme, preferably blended with an appropriate carrier. The method for treating food stuffs comprises treating the food stuffs with an anti-infection agent comprising an effective amount of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for the bacteria.. Additionally, chimeric lytic enzymes shuffled lytic enzymes, and holin proteins, either alone or in combination, may be used to treat or prevent bacterial contamination of foodstuffs. The lytic enzyme can be used for the treatment or prevention of various strains of Staphylococcus, Streptococcus, Listeria, Salmonella, E. coli, Campylobacter, Pseudomonas, Brucella, other bacteria, and an,Y combination thereof. Feed for livestock, poultry and beef in slaughterhouses, canned and bottled goods, salad bars, and eggs are just some of the food items that can be treated with at least one lytic enzyme to reduce the risk of food contamination by bacteria.

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What we claim is:

1) A method for the prevention of food poisoning, comprising

administering to a food stock:

an effective amount of at least one enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria wherein said at least one enzyme is selected from the group consisting of lytic enzymes, shuffled lytic enzymes, chimeric lytic enzymes, and combinations thereof;

wherein said food stock is selected from the group consisting of live stock feed, eggs, salad bars, beef carcasses, chicken carcasses, food to be canned, and livestock feed.

2) The method of claim 1, wherein said food stock is livestock feed.

3) The method of claim 2, wherein said livestock feed is for the feeding of cattle.

4) The method of claim 2, wherein said livestock feed is for the feeding of chickens.

5) The method of claim 2, wherein said livestock feed is for the feeding of hogs.

6) The method of claim 2, wherein said livestock feed is for the feeding of sheep.

7) The method of claim 2, wherein said livestock feed is dry.

8) The method of claim 2, wherein said livestock feed is a slurry.

9) The method of claim 1, further comprising delivering said at least one enzyme in a carrier suitable for delivering said at least one said enzyme.

10) The method according to claim 1, wherein said at least one enzyme is specific for at least one strain of *Pseudomonas.*

11) The method according to claim 1, wherein said at least one enzyme is specific for *Streptococcus pneumoniae.*

12) The method according to claim 1, wherein said at least one enzyme is specific for *Streptococcus fasciae*

13) The method according to claim 1, wherein said at least one enzyme is specific for at least one strain of *Listeria.*

14) The method according to claim 1, wherein said at least one enzyme is specific for at least one strain of *Salmonella.*

15) The method according to claim 1, wherein said at least one enzyme is specific for at least one strain of *E. coli.*

16) The method according to claim 1, wherein said at least one enzyme is specific for at least one strain of *Campylobacter.*

17) The method according to claim 1, wherein said at least one enzyme is specific for at least one strain of *Pseudomonas*.

18) The method according to claim 1, wherein said at least one enzyme is specific for *Streptococcus mutans*.

19) The method according to claim 1, wherein said at least one enzyme is specific for *Mycobacterium tuberculosis*.

20) The method according to claim 1, wherein said at least one enzyme is specific for at least one strain of *Streptococcus*.

21) The method according to claim 9, wherein said carrier is selected from the group consisting of water, oil, micelles, inverted micelles, liposomes, starches, carbohydrates, and combinations thereof.

22) The method according to claim 1, wherein said at least one enzyme is in an environment having a pH which allows for activity of said at least one enzyme.

23) The method according to claim 1, wherein said at least one enzyme is in a buffer that maintains pH of the composition at a range between about 4.0 and about 9.0.

24) The method according to claim 23, wherein said buffer maintains the pH of the composition at the range of between about 5.5 and about 7.5.

25) The method according to claim 23, wherein said buffer comprises a reducing agent.

26) The method according to claim 25, wherein said reducing agent is dithiothreitol.

27) The method according to claim 23, wherein said buffer comprises a metal chelating reagent.

28) The method according to claim 27, wherein said metal chelating reagent is ethylenediaminetetraacetic disodium salt.

29) The method according to claim 23, wherein said buffer is a citrate-phosphate buffer.

30) The method according to claim 1, further comprising a bactericidal or bacteriostatic agent as a preservative.

31) The method according to claim 1, wherein said at least one enzyme is present in an amount ranging from about 100 to about 500,000 units per milliliter.

32) The method according to claim 31, wherein said at least one enzyme is present in an amount ranging from about 1,000 units to about 100,000 units per milliliter.

33) The method according to claim 32, wherein said at least one enzyme is present in an amount ranging from about 10,000 units to about 100,000 units per milliliter.

34) The method according to claim 1, wherein said food stock is a salad bar, comprised of salad.

35) The method according to claim 9, wherein said at least one enzyme is administered by spraying said at least one enzyme onto said salad..

36) The method according to claim 35, wherein said carrier for spraying said at least one enzyme onto said salad is selected from the group consisting of water and an oil based mixture.

37) The method according to claim 35, wherein said at least one enzyme is contained in a protecting structure selected from the group consisting of a micelle, reverse micelle, liposome, and combinations.

38) The method according to claim 34, wherein said at least one enzyme is administered by dusting said at least one enzyme onto said salad.

39) The method according to claim 9, wherein said at least one said enzyme is applied to carcasses of animals in a slaughterhouse processing plant.

40) The method according to claim 38, wherein said animals are selected from the group consisting of cattle, hogs, sheep, and chickens.

41) The method according to claim 39, further comprising administering said at least one enzyme by dipping said carcasses of said animals into a liquid containing said at least one enzyme.

42) The method according to claim 39, further comprising administering said at least one enzyme by dusting said at least one enzyme onto the carcasses of said animals in the slaughterhouse processing plant.

43) The method according to claim 9, wherein said at least one enyzme is added during grinding of ground meat.

44) The method according to claim 43, wherein said ground meat is ground beef.

45) The method according to claim 43, wherein said carrier carrying said at least one enzyme is a liquid carrier.

46) The method according to claim 43, wherein said carrier carrying said at least one enzyme is in the form of a powder, said powder being selected from the group selected from a carbohydrate powder, a cornstarch powder, and a protein powder.

47) The method according to claim 9, wherein said at least one enzyme is added to ground meat after said meat is ground.

48) The method according to claim 9, wherein said food stock is at least one egg.

49) The method according to claim 48, wherein said carrier is a liquid, and said at least one egg is dipped into said liquid.

50) The method according to claim 48, wherein said carrier is a liquid, and said liquid containing said at least one enzyme is sprayed on said at least one egg.

51) The method according to claim 48, wherein said carrier is a powder, and said powder containing said at least one enzyme is sprinkled on said at least one egg.

52) The method according to claim 48, wherein said carrier is a powder, and said egg is rolled in said powder.

53) The method according to claim 1, wherein said at least one enzyme is added to a closed container containing said food stock, said at least one enzyme being added prior to said container being closed during food processing.

54) The method according to claim 53, wherein said closed container is a bottle.

55) The method according to claim 53, wherein said closed container is a can.

56) The method according to claim 53, wherein said at least one enzyme is lyophilized.

57) The method according to claim 53, further comprising a carrier suitable for delivering

said at least one enzyme.

58) The method according to claim 57, wherein said carrier is selected from the group consisting of water, emulsion, and a solution.

59) The method according to claim 53, wherein said at least one enzyme is protected by a structure selected from the group consisting of micelles, liposomes, and inverted micelles.

60) The method according to claim 53, further comprising a buffer that maintains pH of the composition at a range between about 4.0 and about 9.0.

61) A method for the treatment and prevention of food contamination, comprising: administering to a surface where food resides an effective amount of at least one enzyme selected from the group consisting of lytic enzymes, shuffled lytic enzymes, chimeric lytic enzymes, and combinations thereof, and combinations thereof.

62) The method according to claim 61, further comprising a carrier suitable for delivering said at least one lytic enzyme.

63) The method according to claim 62, wherein said carrier is selected from the group consisting of water, emulsion, and a solution.

64) The method according to claim 62, wherein said carrier is applied to said surface with a

cloth.

65) The method according to claim 62, wherein said carrier is applied to said surface with a sponge.

66) The method according to claim 62, wherein said carrier is sprayed on said surface.

67) A method for treating animal feed to prevent or treat bacterial contamination, comprising, administering to said animal feed an effective amount of at least one enzyme selected from the group consisting of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria, at least one modified version of said at least one lytic enzyme, and combinations thereof, wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled enzymes, chimeric enzymes, holin enzymes, and combinations thereof.

68) The method of claim 67, wherein said animal feed is for an animal selected from the group consisting of cattle, chickens, hogs and sheep.

69) The method of claim 67, wherein said animal feed is dry.

70) The method of claim 67, wherein said animal feed is a slurry.

71) The method of claim 67, further comprising delivering said at least one lytic enzyme in a carrier suitable for delivering said at least one lytic enzyme.

72) A bacterial resistant animal feed comprising:

an animal feed; and

an effective amount of at least one enzyme selected from the group consisting of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria, at least one modified version of said at least one lytic enzyme, and combinations thereof, wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, holin enzymes, and combinations thereof.

73) The bacterial resistant animal feed of claim 72, further comprising a carrier, wherein said at least one enzyme is in said carrier.

74) The method for treating salad bars to prevent or treat bacterial contamination, comprising: administering to said salad of said salad bar an effective amount of at least one enzyme selected from the group consisting of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria, at least one modified version of said at least one lytic enzyme, and combinations thereof, wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, holin enzymes, and combinations thereof.

75) The method of claim 74, further comprising delivering said at least one enzyme in a carrier suitable for delivering said at least one lytic enzyme.

76) A bacterial resistant salad bar comprising:

salad in a salad display in a public area; and

an effective amount of at least one enzyme selected from the group consisting of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria, at least one modified version of said at least one lytic enzyme, and combinations thereof, wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, holin enzymes, and combinations thereof.

77) The bacterial resistant salad bar of claim 76, further comprising a carrier, wherein said at least one enzyme is in said carrier.

78) A method for treating carcasses of animals to prevent food poisoning, comprising:

administering to said carcasses of said animals an effective amount of at least one enzyme selected from the group consisting of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria, at least one modified version of said at least one lytic enzyme, and combinations thereof, wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled enzymes, chimeric enzymes, holin enzymes, and combinations thereof. to said carcasses of said animals.

79) The method according to claim 78, wherein said animals are selected from the group consisting of cattle, hogs, sheep, and chickens.

80) The method according to claim 78, further comprising dipping said carcasses of said

animals into a liquid containing said at least one enzyme.

81) The method according to claim 80, further comprising dusting said at least one enzyme onto the carcasses of said animals in the slaughterhouse processing plant.

82) A method for treating ground meat to prevent food poisoning, comprising: administering to said ground meat an effective amount of at least one enzyme selected from the group consisting of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria, at least one modified version of said at least one lytic enzyme, and combinations thereof, wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, holin enzymes, and combinations thereof. to said carcasses of said animals .

83) The method of claim 82, wherein said at least one enzyme is added during the grinding of ground meat.

84) The method of claim 81, wherein said enzyme is in a carrier suitable for delivering said at least one enzyme.

85) The method of claim 82, wherein said carrier is selected is selected from the group consisting of water, oil, micelles, inverted micelles, liposomes, starches, carbohydrates, or combinations thereof.

86) A bacteria resistant ground meat, comprising:

ground meat; and

an effective amount of at least one enzyme selected from the group consisting of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria, at least one modified version of said at least one lytic enzyme, and combinations thereof, wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, holin enzymes, and combinations thereof.

87) The bacteria resistant ground meat of claim 86, wherein said ground meat is ground beef.

88) The bacteria resistant ground meat of claim 86, wherein said bacteria for which said enzyme is specific is *E. coli*.

89) The bacteria resistant ground meat of claim 86, further comprising a carrier, wherein said at least one enzyme is in said carrier.

90) The bacteria resistant ground meat of claim 89, wherein said carrier is selected from the group consisting of water, oil, micelles, inverted micelles, liposomes, starches, carbohydrates and combinations thereof.

91) A method for treating eggs to prevent food poisoning, comprising administering to shells of said eggs an effective amount of at least one enzyme selected from the group consisting of at least one lytic enzyme produced by a bacteria infected with a

bacteriophage specific for said bacteria, at least one modified version of said at least one lytic enzyme, and combinations thereof, wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, holin enzymes, and combinations thereof. to said carcasses of said animals.

92) The method of claim 91, further comprising a carrier suitable for delivering said at least one enzyme to the shells of said egg.

93) The method of claim 92, wherein said eggs are dipped in a solution comprising said at least one enzyme.

94) The method of claim 92, wherein said shells of said eggs are dusted with a carrier comprising said at least one enzyme.

95) The method of claim 91, wherein said carrier is selected from the group consisting of water, oil, micelles, inverted micelles, liposomes, starches, carbohydrates, and combinations thereof.

96) A method for reducing bacterial infections of sealed food containers, comprising administering to said food containers before they are sealed an effective amount of at least one enzyme selected from the group consisting of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria, at least one modified version of said at least one lytic enzyme, and combinations thereof, wherein said

modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, holin enzymes, and combinations thereof. to said carcasses of said animals.

97) The method of claim 96, wherein said sealed food container is a bottle.

98) The method of claim 96, wherein said sealed food container is a can.

99) The method of claim 96, further comprising delivering said enzyme in a carrier suitable for delivering said enzyme.

100) The method of claim 99, wherein said carrier is selected from the group consisting of a micelle, reverse micelle, liposome, and combinations thereof.

101) The method according to claim 96, wherein the enzyme is in an environment having a pH which allows for activity of said enzyme.

102) The method according to claim 101, wherein said enzyme is in a buffer that maintains pH of the composition at a range between about 4.0 and about 9.0.

103) The method according to claim 103, wherein said buffer maintains the pH of the composition at the range of between about 5.5 and about 7.5.

104) The method according to claim 102, wherein said buffer comprises a reducing agent.

105) The method according to claim 104, wherein said reducing agent is dithiothreitol.

106) The method according to claim 102, wherein said buffer comprises a metal chelating reagent.

107) The method according to claim 106, wherein said metal chelating reagent is ethylenediaminetetraacetic disodium salt.

108) The method according to claim 102, wherein said buffer is a citrate-phosphate buffer.

109) The method according to claim 96, further comprising a bactericidal or bacteriostatic agent as a preservative.

110) The method according to claim 96, wherein said at least one enzyme is present in an amount ranging from about 100 to about 500,000 units per milliliter.

111) The method according to claim 110, wherein said at least one enzyme is present in an amount ranging from about 1,000 units to about 100,000 units per milliliter.

112) The method according to claim 111, wherein said at least one enzyme is present in an amount ranging from about 10,000 units to about 100,000 units per milliliter.

113) A method for reducing bacterial infections of liquids, comprising administering to said

liquids an effective amount of at least one enzyme selected from the group consisting of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria, at least one modified version of said at least one lytic enzyme, and combinations thereof, wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, holin enzymes, and combinations thereof.

114) The method of claim 113, wherein said liquid is in a bottle.

115) The method of claim 113, wherein said liquid is in a can.

116) The method of claim 113, further comprising delivering said enzyme in a carrier suitable for delivering said enzyme.

117) The method of claim 116, wherein said carrier is selected from the group consisting of a micelle, reverse micelle, liposome, and combinations thereof.

118) The method according to claim 113, wherein the enzyme is in an environment having a pH which allows for activity of said enzyme.

119) The method according to claim 118, wherein said enzyme is in a buffer that maintains pH of the composition at a range between about 4.0 and about 9.0.

120) The method according to claim 119, wherein said buffer maintains the pH of the

composition at the range of between about 5.5 and about 7.5.

121) The method according to claim 119, wherein said buffer comprises a reducing agent.

122) The method according to claim 121, wherein said reducing agent is dithiothreitol.

123) The method according to claim 119, wherein said buffer comprises a metal chelating reagent.

124) The method according to claim 123, wherein said metal chelating reagent is ethylenediaminetetraacetic disodium salt.

125) The method according to claim 119, wherein said buffer is a citrate-phosphate buffer.

126) The method according to claim 113, further comprising a bactericidal or bacteriostatic agent as a preservative.

127) The method according to claim 113, wherein said at least one enzyme is present in an amount ranging from about 100 to about 500,000 units per milliliter.

128) The method according to claim 127, wherein said at least one enzyme is present in an amount ranging from about 1,000 units to about 100,000 units per milliliter.

129) The method according to claim 128, wherein said at least one enzyme is present in an

amount ranging from about 10,000 units to about 100,000 units per milliliter.

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 38228-0002	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US01/42886	International filing date (*day/month/year*) 01 November 2001 (01.11.2001)	(Earliest) Priority Date (*day/month/year*) 02 November 2000 (02.11.2000)
Applicant NEW HORIZONS DIAGNOSTICS, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the Report**
   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
   ☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:
   ☐ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☐ furnished subsequently to this Authority in computer readable form.
   ☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.
2. ☐ **Certain claims were found unsearchable** (See Box I).
3. ☐ **Unity of invention is lacking** (See Box II).
4. With regard to the **title**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:
5. With regard to the **abstract**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.
6. The figure of the **drawings** to be published with the abstract is Figure No. ____
   ☐ as suggested by the applicant. ☒ None of the figures
   ☐ because the applicant failed to suggest a figure.
   ☐ because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/42886

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 38/43; C12S 3/00; C12N 9/36; A23L 3/3463
US CL : 424/94.6; 435/259, 267, 206, 195; 426/321, 335
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/94.6; 435/259, 267, 206, 195; 426/321, 335

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,763,251 A (GASSON) 09 June 1998 (09.06.1998), see entire document.	1-129
X	WO 95/31562 A1 (QUEST INTERNATIONAL B.V.) 23 November 1995 (23.11.1995), see entire document.	1-129
Y, P	US 6,159,688 A (BORCHERT et al) 12 December 2000 (12.12.2000), see entire document.	1-129
Y	WITTE, A. et al. Characterization of Escherichia coli lysis using a family of chimeric E-L genes. FEMS Microbiology Letters, 1998, Vol. 164, pages 159-167, see entire	1-129
Y	DIAZ, E. et al. Chimeric Pneumococcal Cell Wall Lytic Enzymes Reveal Important Physiological and Evolutionary Traits. The Journal of Biological Chemistry. 25 March 1991, Vol. 266, No. 9, pages 5464-5471, see entire document.	1-129

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 October 2002 (24.10.2002)	12 NOV 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Francisco C Prats Telephone No. 703-308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

Continuation of B. FIELDS SEARCHED Item 3:
USPAT; USPG-PUB, EPO; JPO, DERWENT, CAPLUS; search terms: enzyme, lytic, lysis, phage, virus, food, feed, holin, lysin, chimeric, shuffled